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Three areas of research were implemented experimentally in the summer of 1992. (1) further description of calcium microdomains and their role in synaptic transmission; (2) a morphological analysis of rat synaptic vesicles injected into presynaptic terminal of the squid; and (3) the effect of Brefilden A (BFA) on the distribution and size of synaptic vesicles.

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Technical Report for AFOSR 92-J-0363DEF

R. Llinás and W. Berry

"Synaptic Transmitter Release"

June 1, 1992- May 31-1993

Three areas of research were implemented experimentally in the summer of 1992: (1) further description of calcium microdomains and their role in synaptic transmission; (2) a morphological analysis of rat synaptic vesicles injected into presynaptic terminal of the squid; and (3) the effect of Brefilden A (BFA) on the distribution and size of synaptic vesicles.

Calcium microdomains

Concerning calcium microdomains, the demonstration of existence of this calcium concentration compartment has made a substantial impact on both neuroscience and the cellular biological fields. Indeed the paper published in *Science* in 1992 (enclosed) has been one of the most requested in the history of our lab and I have received copies of many papers submitted and in press that build on that concept.

Last summer we began to determine more precisely the distribution of kinetics of these microdomains, utilizing single action potentials as opposed to the averaged potentials described in our *Science* paper. Although the experiments were very difficult to generate the results were promising as we could observe for the first time microdomains generated by a single action potential. Even under these conditions, well-defined calcium entry at the active zones could be visualized with great detail using a photon-counting camera and a very high optical aperture lens. The experiments were performed under conditions where action potentials were enhanced in the duration by the presence of tetraethyl ammonium. As in previous results synaptic release was enhanced by the appearance of microdomains, which we have referred to as "quantum emission domains." These QEDs had a similar distribution to the averaged picture but were smaller in number.

The second important measurements relating to this have been the utilization of dynamic measurements to increase speed with which such microdomains are formed and disappear. To that effect we have decided to move from N-aequorin-j to more sensitive aequorins which would have

higher resolution in time, and we began to implement a new technique using high speed video cameras. While only preliminary results could be obtained we are now poised to continue such research in 1993.

Morphological analysis of rat synaptic vesicles

With regard to analysis of intracellularly injected synaptic vesicles, we injected both pre- and post-synaptic elements and have been imaging the distribution and size of vesicles using electron microscopy in collaboration with Reiner Martin in Ulm, Germany. The results suggest that rat vesicles injected intracellularly are recognized and indeed released during depolarization, validating the vesicle hypothesis. We are at this moment reviewing data with the aim of publishing a paper on the matter.

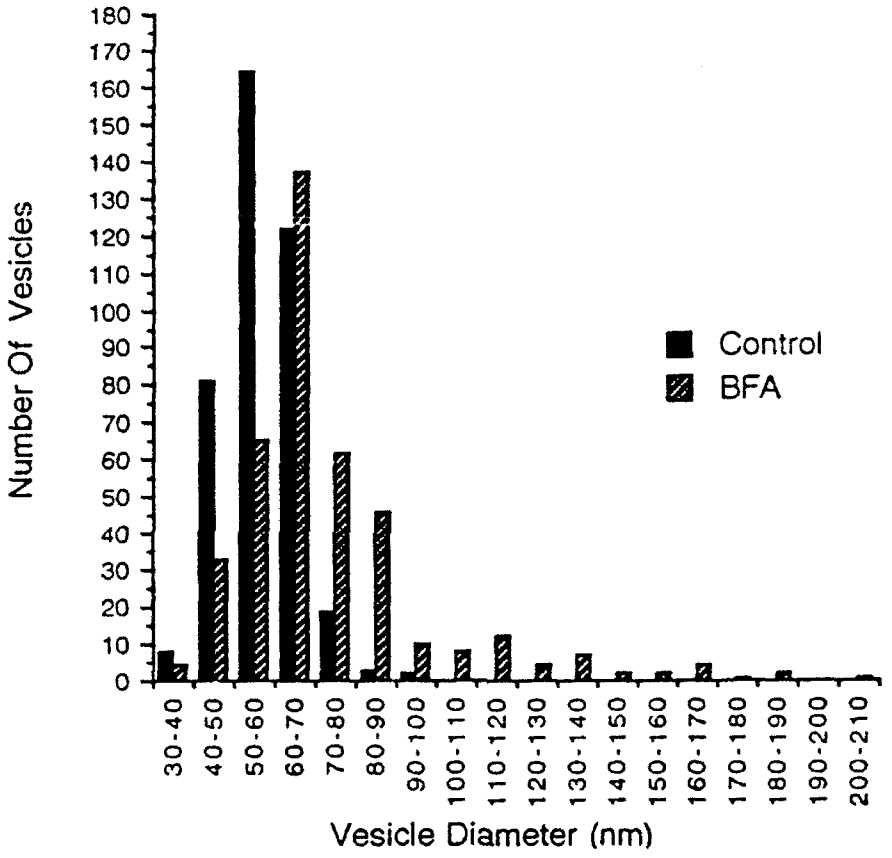
Effect of Brefilden A *on the distribution and size of synaptic vesicles*

The third area of research relates to the effect of BFA on the distribution and size of synaptic vesicles in the squid synapse. Adding BFA to the bath and determining the speed with which synaptic transmission is modified, to our delight, we found it is blocked by this substance and electron microscopical examination in the BFA of synaptic vesicles is modified as illustrated in Fig. 1A and table 1 (Fig. 1B). Indeed the mean diameter of the vesicles was modified from 55.3 ± 2 to 71.8 ± 3.5 nM.

Because of the clear response obtained under the circumstances we can say that the synaptic vesicle pathway belongs to the structural branch of membrane exocytosis. This is a significant finding as it associates synaptic transmission with growth of terminals as proposed many years ago (Llinás et al.); that "isometric growth" is a mechanism for synaptic release. An abstract was sent to the Society of Cell Biology for their 1993 meeting.

A

Distribution of rat synaptic vesicle diameters in the presence and absence of BFA.



B

Table N. Effect of Brefiden A on Rat Synaptic Vesicle Diameter

Treatment	N*	Mean vesicle diameter(nm) + SE
Control	8	55.3 + 2.0
BFA	8	71.8 + 3.5